THE GUM FERMENTATION OF SUGAR CANE JUICE.

By R. Greig Smith, M.Sc., Macleay Bacteriologist to the Society.

(Plate xxx.)

During the process of manufacturing sugar in the cane-mills, the juice frequently becomes more or less viscous or "gummy," and when this takes place the crystallisation of the sugar is considerably hindered. The "gumming" may occur in the cane juice—the immediate product of the crushed cane—or it may develop at any stage in the manufacture of the raw sugar, especially if the juice or syrup is allowed to cool, or to stand for any length of time. Experience has shown that the only way to minimise the trouble is to complete the crystallisation of the sugar as quickly as possible. The cause of the formation of the gum is not definitely known, although the prevailing idea is that it is developed from something which is contained in the juice.

I received two samples of gummed cane juice from Mr. T. Steel of the Colonial Sugar Company, and was at the same time informed that any information regarding the properties of micro-organisms, which might induce the gummy fermentation of sugar, would be of the greatest economic importance to the sugar manufacturers. Each of the samples consisted of about 5 c.c. of roller cane juice, which had not been treated in any way. One of the samples was viscid; the other appeared limpid, but I was informed that, as compared with normal cane juice, it was decidedly gummy.

The Separation of the Gum-forming Organism.

From the samples several organisms were separated, but none of them produced any apparent viscosity in solutions containing

10 % saccharose, that is to say, the solutions did not become ropy and capable of being drawn into threads as in the case of fluid cultures of many slime-forming organisms. The bacteria were further cultivated in nutrient agar with 20 % saccharose. One of the organisms formed raised transparent mucilaginous colonies, which made it appear probable that it was the most likely organism to produce "gum." This became more probable when short viscous threads were formed on raising the cover from a Petri dish, in which a pure culture had covered the surface of the agar and grown up the rather low side of the dish. A gummy substance had apparently been formed by the bacterium, but since no apparent viscosity had been produced in saccharose fluid media, it was desirable to test the fluid cultures more rigorously for viscosity.

Viscosity Produced in Solutions of Saccharose.

With this object in view, an infusion was prepared from 1,000 grms. of grass and 1,000 c.c. of tap water, and to this 200 grms. of cane sugar were added. A 500 c.c. sterile portion was inoculated with the organism and incubated at 28° C., with a check 500 c.c. test. On the third day a viscous film was observed floating upon the surface of the infected medium, and partly adhering to the glass of the culture flask. On the fifth day the culture, with its floating zoogloea films, was boiled, and during the process it was observed to foam very much, as if the solution contained carbon dioxide, while the control test, on being similarly treated, boiled quietly. Both fluids were then filtered through paper; the culture filtered slowly, and the zoogloea films were retained on the filter. The fluids were brought to a uniform temperature (24° C., the air temperature being 22° C.), and allowed to run from a 100 c.c. pipette, provided with a narrow outlet, and having two marks on the stem, one above and the other below the bulb. The time taken by the surface of the fluids in passing from the upper to the lower mark was noted. For purposes of comparison, the viscosity of other solutions was determined in the same apparatus and at the same temperature.

Viscosity of the Culture Compared with other Solutions.

	Time in Seconds.	Viscosity ratio. Water = 100.
Culture in grass infusion with 20 % sugar, filtered	675	293
Grass infusion with 20 % sugar, filtered... ...	290	126
Grass infusion with 20 % sugar and 2 % dextrin	310	135
Grass infusion with 20 % sugar and 1 % starch, filtered	355	154
Grass infusion with 20 % sugar and 2 % starch, not filtered	625	271
Distilled water	230	100

It is at once evident that the organism has produced a decided viscosity in the liquid medium, and had the culture not been filtered, the viscosity would have been greater. The organism is, therefore, capable of producing a viscous substance in nutrient solutions containing cane sugar.

The bacterium was subsequently grown in a saline medium containing 10 % saccharose and 0·1 % peptone. The viscosity of a three weeks' culture was determined, but the temperature was lower (18° C.), and the apparatus was probably different from that used before, although the volume was about the same, viz., 100 c.c.

	Time in Seconds.	Viscosity Ratio.
Three weeks' culture at 37° C.	530	189
Culture medium	303	108
Distilled water	280	100

The Nature of the Viscous Substance.

To obtain some idea of the nature of the viscous substance, a portion of the filtered culture was treated with alcohol, when amorphous flakes were precipitated. These adhered tenaciously to the glass vessel in which the precipitation was conducted, and were easily washed with fresh alcohol. When treated with water the flakes appeared to partly dissolve and partly swell up; on

boiling, an apparent solution was obtained. The gum was precipitated by alcohol, and redissolved in water several times until a comparatively white precipitate, free from sugar, was obtained. The aqueous solution was easily precipitated by alcohol.

On warming with dilute tartaric acid, a reducible sugar was formed (saccharose treated similarly was not inverted). These tests show that the viscous substance is of the nature of dextran or fermentation gum, but the identification was deferred until a greater quantity had been prepared from a medium containing a more definite substance than grass infusion, some of the carbohydrates of which would be precipitated with the gum.

The Fermentation of Saccharose.

The action of the organism, which had by this time been found to be a sporulating rod or bacillus, is of considerable importance, for it appears to be an undoubted fact that the gum is formed from the sugar. There may, also, be a further loss of saccharose from the formation of hexoses, if the organism secretes invertase. To test these points, a culture medium containing the following constituents was prepared.

Saccharose	100 grm.
Potassium chloride	5 „
Sodium phosphate...	2 „
Peptone	1 „
Tap water	1000 c.c.

Sterile litre portions of this medium were infected with large loops of an agar culture of the bacillus, and incubated at 22°, 28° and 37° C. respectively. The cultures soon became white and opalescent like dilute separated milk. A thin film formed on the surface, and when the flasks were allowed to stand without shaking, a layer about a centimetre thick of a mucilaginous or starch paste-like substance formed at the bottom of the liquid. When this was removed by continued shaking, it remained suspended in the milky medium. The layer, when undisturbed, disappeared on continued incubation. The culture fluid contained

gum, unaltered saccharose, and a reducing sugar or mixture of sugars. A small quantity of acid was also formed. In estimating the sugars, it is necessary to remove the gum, and to do this various precipitants or coagulants were tried. Basic acetate of lead was found to be useless, as a diffuse emulsion was formed which refused to coagulate. The addition of milk of lime to the lead emulsion produced coagulation, but an equally good coagulation was obtained by the use of milk of lime alone. Lime is not an ideal coagulant on account of the possible formation of difficultly soluble compounds with the sugars, especially with levulose. The percentages of saccharose inverted and not inverted might not, therefore, be a true index of the rate of change when lime is employed, and a number of analyses bore out this contention. A coagulation of the gum was also attempted with anhydrous magnesium sulphate and with calcined magnesia, but these magnesium compounds were found to be quite inert—no coagulation was obtained, and the filtrate appeared similar to the solution before the addition. Acid mercuric nitrate, like basic lead acetate, formed a diffuse emulsion which passed slowly and without retention through filter paper. The neutralisation of the acid with sodium hydrate, until the emulsion became yellowish, was also without effect.

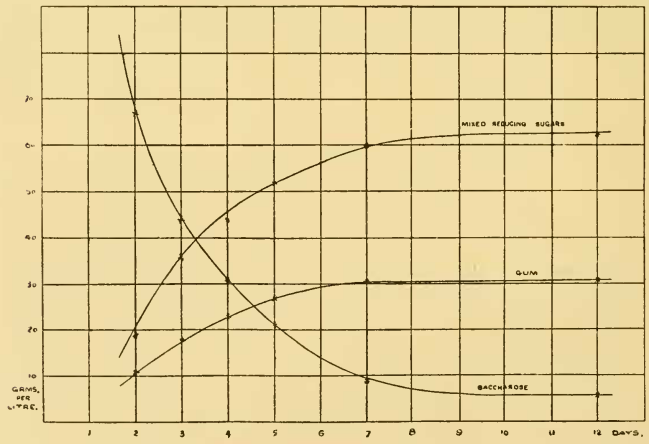
The only safe coagulant appears to be alcohol, and in the following work upon the action of the bacillus this was used. After many trials the following method was adopted. Twenty c.c. of the culture is slowly dropped into 60 c.c. of strong alcohol (methylated spirit distilling at 77·5 – 78·5° C.) while the latter is being vigorously stirred. A drop of phenolphthalein solution is next added, and the acidity neutralised with dilute sodium hydrate. Finally 20 to 40 c.c. of alcohol are added to make certain that coagulation has been complete. After standing for two or three hours, the gum is filtered off upon a dry and tared filter and scraped from the beaker, to which it adheres somewhat firmly. If necessary, the adhering particles are treated with a small quantity of hot water, the gum precipitated with excess of alcohol and filtered. The gum is dried at 100° C. until of

constant weight, and finally the ash is determined. On subtracting the ash from the dried gum, the amount of crude gum in the portion taken is obtained. The alcohol is distilled off from the sugars, which are estimated volumetrically before and after inversion with hydrochloric acid at 70° C.

The following table shows the progressive formation of gum and mixed reducing sugars in the dilute peptone solution. The percentages are given to the nearest whole number.

THE FERMENTATION OF SACCHAROSE AT 37° C. IN A SOLUTION CONTAINING 100 GRMS. SACCHAROSE, 1 GRM. PEPTONE AND SALTS PER LITRE.

Time in days	0	2	3	4	5	7	12
Saccharose	100	67	44	31	21	9	6
Mixed reducing sugars ..	—	19	36	44	52	60	62
Crude gum	—	11	18	23	27	31	31



CURVES SHOWING THE LOSS OF SACCHAROSE AND FORMATION OF GUM AND INVERT SUGAR

The formation of gum and the inversion of the sugar are seen to go on steadily from the second day until a balance is practically established between the constituents on the seventh day. The saccharose is not entirely inverted, nor is this to be expected. Marshall Ward and Reynolds Green* found a complete inversion of saccharose with their sugar bacteria, but it is just possible that this was brought about by the acid fluids (their organism produced in the culture fluid 0·7 % acetic and 0·057 % succinic acids) during the chemical manipulation and not by the invertase secreted by the organism. Even with the small amount of acid in my cultures I noted in my preliminary experiments an increase in the invert sugar when the acids were not neutralised. The acids formed by the bacillus have also a solvent action upon the gum. In one case I obtained 31 grms. per litre of crude gum as against 29 grms. when the acidity was not neutralised during the precipitation with alcohol.

The Influence of varying Amounts of Peptone.

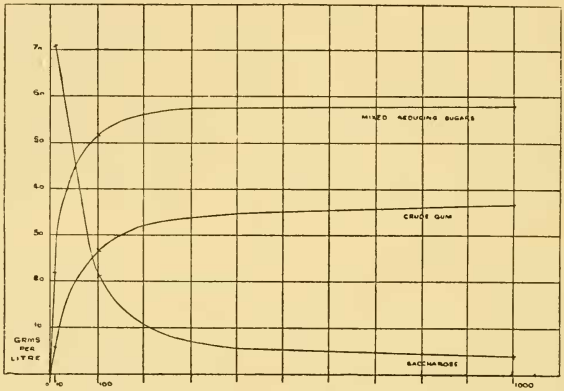
The bacillus can grow with marked action upon saccharose in exceedingly poor nutrient solutions. The solutions in which the action has been already shown contained only $\frac{1}{10}$ % of peptone. With smaller amounts the formation of gum is evident from the appearance of the cultures, although the action is naturally not so rapid. With the object of determining the influence of peptone on the fermentation, a series of cultures containing varying amounts of peptone, but with the other constituents as before, were made and analysed upon the same, viz., the fifth, day. The results expressed in terms of 100 parts of original saccharose, *i.e.*, in parts per litre of culture fluid, are given in the table, and are also plotted upon the curves that follow.

---

* Ward and Green, Proc. Roy. Soc. lxv., No. 414, 79.

THE FERMENTATION OF SACCHAROSE WITH VARYING PERCENTAGES OF PEPTONE.

	Percentage of peptone in culture fluid.				
	None.	0·001	0·01	0·1*	1·0
Saccharose...	99†	97	71	21	4
Mixed Reducing Sugars	0·5‡	2	23	52	58
Crude Gum	0·4	1	6	27	37
Acidity as lactic acid in 100 c.c. of culture	None	0·002	0·03	0·08§	0·18



It is evident from these results that the fermentative activity is considerably influenced by the presence of peptone. But although an increase of growth was expected from an increase of peptone the relative formation of gum and mixed reducing sugars could not have heen foretold. With increasing amounts of peptone there is proportionately more gum than reducing sugars formed, there is more saccharose fermented and more acid formed. With no peptone and with 0·001 % peptone the changes are too small to enable any deduction to be made. The influence of the

* This column is taken from the previous results. † Estimated by difference. ‡ Estimated in the culture directly without previous precipitation of the gum. § Taken from the notes on page 604.

peptone on the formation of gum is better seen on comparing the amounts formed in 0·01 % and 1·0 % solutions; in the former the ratio of gum to reducing sugars is 1 : 3·8, while in the latter it is 1 : 1·6. But since it is probable that the gum is formed from the sugar inverted, we might calculate the ratio between the gum and the sugar wholly inverted, *i.e.*, the saccharose which has disappeared calculated to hexose. With 0·01 % peptone this ratio is 1 : 5·1, and with 1·0 % it is 1 : 2·7. So that in whatever way we look at these two columns we find that an increase of peptone gives an increase of gum and a relative decrease of hexoses, and conversely, with decreasing amounts of nitrogenous material, there is a decrease of gum and relative increase of reducing sugars.

It is to be noted that the original composition of the culture fluid as regards peptone is excellent for the purpose. The best amount would probably be 0·3 or 0·4 %. It was also found incidentally that 10 % of saccharose is about the optimum quantity. With 20 % (and even in the presence of 1 % peptone) the fermentation was far from complete.

The Action upon other Sugars.

In testing the action of the bacillus upon other sugars, solutions similar to those already employed, but containing other sugars in place of saccharose, were used. Dextrose was first tested, and media containing commercial starch glucose were infected and cultivated at 37° and 22° respectively. The organisms grew well in the media and produced a turbidity and a flocculent precipitate. The dextrose was estimated from time to time, and the following numbers were obtained :—

Percentage of Dextrose in Cultures of the Bacillus.

Incubation period.	At 37°.	At 22°.
At start	7·5	9·5
1 day	7·4	—
2 days	7·4	—
4 ,, ...	7·4	—
6 ,, ...	7·3	9·4
15 ,, ...	—	9·2

It is apparent that no dextrose has been utilised in the formation of gum, and indeed this was to be expected from the absence of the opalescent appearance which is so marked in the saccharose cultures. A portion of the 15 days' culture at 22° was treated with alcohol when a slight flocculent non-adhesive precipitate was thrown down. This was estimated and found equal to 0·046 %.

Cultures were also made in solutions containing lactose, levulose and maltose respectively. Alcohol did not throw down a precipitate from the lactose culture, but slight loose, flocculent, non-adhesive precipitates similar to that obtained in the glucose culture were precipitated from the media containing levulose and maltose.

From these tests it is apparent that gum is not formed in solutions of the commonly occurring sugars, and this has been the experience of all who have investigated the action of similar saccharose-gum fermenting bacteria.

The Gum is Probably the Swollen or Diffluent Capsule of the Bacillus.

Whether the gum is formed from the sugar by an enzyme extracellularly, or whether it is the diffluent sheath or capsule of the organism, is difficult to prove absolutely. Happ and also, according to Lehmann and Neumann, Ritsert believed that the gum was formed extracellularly, and did not arise from the swelling of the cell membrane, because the swollen capsule could not be demonstrated microscopically. Marshall Ward and Reynolds Green believed that the gum formed by their bacterium was nothing more than the extremely diffluent walls of the cell.

My own observations upon this bacillus lead me to think that the latter hypothesis is probably correct. The gum is the capsule which has dilated so much that it has ceased to be a capsule, and has become a part of the culture fluid. The dilation or swelling is hastened by the excreted acid, which is thus a protection, or rather assistance, to the organism, inasmuch as the growth is accelerated by the removal of what would otherwise be a hindrance to the free movement of the organism. The gum is easily soluble in dilute mineral acids, and slowly soluble in

organic acids, such as acetic, and I have already shown that it is partly soluble in the acids excreted by the cell. An indication of the diffusive action of the excreted acid is seen in the disappearance of the gummy layer at the bottom of resting fluid cultures already mentioned on page 592.

The addition of peptone to a culture fluid, as a rule, causes a numerical increase of the bacteria. If the gum is the diffluent capsule, then the greater the number of bacteria the more gum will probably be formed compared with the other products. As an increase of peptone does increase the percentage of gum actually and relatively, one is justified in concluding that the most probable source of the gum is the capsule.

On staining the cells by the night-blue method, as used for demonstrating flagella, a structure similar to a capsule is manifest. It is possible that the appearance might result from the method of staining, but it is more than probable that the structure is the capsule.

The Chemical Nature of the Gum.

The gum was precipitated from peptone-saccharose culture by alcohol, and dissolved in water and again precipitated. This was repeated several times, and finally it was allowed to stand under dilute spirit for a week. The gum was then freed from alcohol and tested for reducing sugars. No reduction of Fehling's solution was obtained, and the gum was, therefore, taken as being practically pure. When dried it appears as a pale yellow, partly transparent mass. It forms with water an opalescent semi-solution, and apparently swells up rather than dissolves. From the semi-solution the gum does not separate when allowed to stand for lengthened periods, nor when centrifuged. It does not reduce Fehling's solution, and does not form an osazone with phenylhydrazine. When warmed with dilute sulphuric acid, or with dilute hydrochloric acid, a sugar is produced, which reduces Fehling's solution, and which forms an osazone, having the solubility, appearance and melting point (205°) of glucosazone. The sugar is, therefore, either dextrose, levulose, or a mixture of both these sugars.

Basic lead acetate makes the solution more opaque, but does not coagulate the gum. Lime water gives no precipitate. It has been already shown that it is coagulated by milk of lime, but not by mercuric nitrate, magnesium oxide, or magnesium sulphate. Barium hydrate forms a curdy precipitate, which is decomposed by carbon dioxide. A precipitate is also obtained on adding ammoniacal lead acetate. Dilute sodium hydrate is apparently without effect, but the strong hydrate (10 %) slowly makes the solution clear and limpid. Neither iodine, tannic acid, nor ferric chloride react with solutions of medium strength. When heated in steam for a few hours with dilute nitric acid (2-1), oxalic and tartaric acids are obtained in quantity. Mucic acid is not produced, even when oxidation is effected at lower temperatures.

On heating the dry and powdered gum in capillary tubes, it begins to darken in colour at 160° C. At 183° it shows signs of melting; at 193° the fragments have become soft and adherent, and begin to rise in the capillary tube, apparently the result of decomposition and evolution of water. At 198° the steam bubbles are well marked, and at 200° the gum melts to a transparent brown frothy mass. The brown pyro-substance dissolves readily in water, forming a brown solution like the colour of neutral ferric chloride. It is not precipitated from solution by alcohol at once, although in time a slight sediment settles out. Neither is it precipitated by barium hydrate nor basic lead acetate. It does not reduce Fehling's solution, but reduction is obtained on hydrolysing the body with dilute acids.

The natural gums are substances allied to the carbohydrates; they are of a faintly acid nature and occur combined with alkaline and earthy bases. For example, O'Sullivan has shown that a Gedda gum consists of the calcium, magnesium and potassium salts of four geddic acids.* The gum acids combine with bases to form well defined compounds, the barium one being the most easily prepared.

Following the method recommended by O'Sullivan for the preparation of the gum acid, the bacterial gum was dissolved in

* O'Sullivan, Trans. Chem. Soc. 1891, 1071.

water and dialysed until free from phosphoric acid after which the faintly acid solution was neutralised with baryta water. A very small quantity (less than 1 c.c.) was required, and the solution appeared to be unaltered and when treated with dilute alcohol did not flocculate. A better result was obtained on adding an excess of baryta water, when there formed a bulky curdy precipitate which rapidly settled. From this behaviour it would appear that the bacterial gum is more nearly related to the starches than to the true gums, such as gum arabic. When no further precipitate was formed the compound was rapidly filtered and washed with water, then with alcohol, until the washings were free from barium. The precipitate was dried *in vacuo* over sulphuric acid, then powdered and finally dried at 100° in a current of dry, CO_2-free air under 100-120 mm. pressure. This method differs from O'Sullivan's in the addition of an excess of baryta water, and it seemed possible that a definite compound might not be formed. To test the matter a sample of gum was divided unequally and each portion was acidified, dialysed, precipitated with excess of baryta water, filtered, washed and dried as described above. The barium in a part of each portion was estimated by precipitation with sulphuric acid and the following results were obtained :—

1st portion—1·4915 grms. gave 0·4505 grm. $BaSO_4$ = 19·84% BaO.
2nd portion—1·5765 grms. gave 0·4766 grm. $BaSO_4$ = 19·86% BaO.

These results are very close and undoubtedly prove that when baryta is added in the manner indicated a definite compound is formed. It would also appear that the barium oxide is present in the same proportion as in the compound formed by adding baryta water to starch. Asbóth* found that the starch compound contained 19·97% BaO, and he considered that this agreed fairly well with the formula $C_{24}H_{40}O_{20}BaO$, which theoretically requires 19·10% BaO.

The Origin of the Gum.

Marshall Ward and Reynolds Green in discussing the action of a gum bacterium isolated by them considered it possible that

* Asbóth, Analyst, xii., 138.

the gum, which consisted of a mixture of a dextro-rotatory with an inactive body, might have been derived from the levulose of the inverted saccharose. Boekhout* in investigating the action of *Streptococcus hornensis* found a lævo-rotatory sugar in the residue from the bacterial fermentation and the gum (dextran) produced a dextro-rotatory sugar on hydrolysis. From these facts he concluded that dextrose was the origin of the gum.

In determining the origin of the gum it is necessary to test the optical activity of the gum and the amount of the residual sugars after the fermentation. This necessitates the use of a polarimeter, and as I had not that instrument in the laboratory I asked Mr. Steel and he consented to do the necessary determinations from material which I supplied. Mr. Steel was so interested with the gum that he investigated it very fully in the endeavour to identify it, and his results, which confirm and amplify my work upon the gum, are embodied in a paper which is published simultaneously with this. From it I extract a few notes bearing upon the question of the origin of the gum.

The gum is lævorotatory ($A_D = -40°$) and the amount of the residual sugars and of the gum show that the latter is formed from the nascent levulose and also from "something else," because the amount of levulose which has disappeared does not equal the gum that is formed. From this we can only conclude that the "something else" must be dextrose. The fact that the gum is lævorotatory and on hydrolysis yields pure levulose does not conflict with the levulose-dextrose origin of the gum. Much more levulose than dextrose is utilised in its formation, and it is quite conceivable that the optical activity of the major constituent may influence the rotatory power of the derived gum. We can convert dextrose to levulose by forming phenyl-glucosazone, which upon reduction and subsequent treatment with nitrous acid yields levulose. Since this change can be accomplished in the laboratory by the formation of intermediate compounds, there is no reason why a gum which yields pure levulose on hydrolysis should not have been derived in part (or entirely) from dextrose.

* Boekhout, Cent. f. Bakt., 2te Abt., vi., 161.

The Affinities of the Gum.

From the reactions and the lævo-rotatory power both of the gum and the sugar resulting from its hydrolysis, the gum appears to be most nearly related to levulan and to inulin, but there are many characters which distinguish it from these. It is also different from the derivatives of inulin, viz., pyro-inulin, met-inulin and levulin, as well as from sinistrin, triticin, secalose, and myco-inulin.

From a review of the lævo-rotatory gummy substances that are hydrolysed to levulose, it appears that this bacterial gum has not hitherto been described. I therefore propose for it the name *levan*, which was suggested by the polariscopic nature of the gum and derived glucose, and also from the fact that another bacterial gum, which is derived from dextrose, and which yields dextrose on hydrolysis, is known as dextran.

The Production of Carbon Dioxide.

During the evaporation of the cultures, a number of small bells formed upon the surface as if a gas were evolved during the process. The most probable gas is carbon dioxide, and to test for it a flask fitted with a rubber cork and lead-away tube was completely filled with a culture, inverted and heated in boiling water. The culture driven from the flask by its expansion, and by the liberation of the dissolved gases, passed through the tube in the cork and was led away into a vessel. The gases which collected in the flask were transferred to a tube and roughly measured before and after absorption with caustic soda. From 300 c.c. of culture fluid, 7·5 c.c. of gas were obtained, and of this 5 c.c. were absorbed by the alkali. It is evident that carbon dioxide is evolved during the growth of the organism. As, however, the test as described above was merely qualitative, and as the gas might be formed in quantity, a quantitative test was made. For this purpose 100 c.c. of culture fluid containing the usual amounts of saccharose and salts, but with 1 % of peptone, were placed in a small Erlenmeyer flask and connected with three other similar flasks, each containing 100 c.c. of clear baryta

water. The inlet tube of the culture flask was fastened with a clip, and to the outlet tube of the third baryta flask a tube containing soda lime was attached to prevent the inward passage of atmospheric carbon dioxide. A current of air was sucked through the apparatus daily after attaching a soda lime tube to the inlet tube of the culture flask. The evolved gas was thus daily removed from the culture. On the fifth day the baryta solutions were added together and rapidly filtered, the barium carbonate was washed and then suspended in water. After adding phenolphthalein, 20 c.c. of standard acid were run in, and the solution boiled to expel the displaced carbon dioxide. The excess of acid was then determined, and by difference the carbon dioxide equivalent obtained.

11·6 c.c. $^{N}/_{2}$ acid used to decompose the barium carbonate
= 0·1276 grm. CO_2 from 10 grms. sugar
= 1·28 grms. CO_2 from 100 grms. sugar.

From the determination it is seen that a considerable quantity of carbon dioxide is formed during the fermentation.

The Production of Acid.

When the cultures were tested with litmus they were found to be distinctly acid, and when small cultures (10 c.c.) were titrated, quantities of $^{N}/_{5}$ alkali varying from 0·3 to 0·6 c.c. were required to neutralise the acidity to phenolphthalein. It was also noted that old cultures contained less acid than comparatively young ones, a circumstance which pointed to the volatilisation of a volatile acid. In connection with this it may be mentioned that the cultures, and also the air of the incubator containing the cultures, had a faint not unpleasant cheesy smell.

During the cultivation at 22° of a two-litre culture, 10 c.c. portions were abstracted and tested. By the second day the acidity was equal to 0·4 c.c. $^{N}/_{5}$ alkali, and it slowly rose to 0·45 c.c. on the tenth day. This is equivalent to from 0·07 to 0 08 % lactic acid.

A number of 50 c.c. portions of saccharose media were infected with several races of the bacillus, and tested after incubation for a week at 37°, with the following results.

PRODUCTION OF ACID BY RACES GROWN FOR SEVEN DAYS AT 37°.

Source.	Race.	C.c. N/5 alkali required to neutralise 50 c.c.	Lactic acid %.
Roller cane juice... ...	O, 1st transfer	2·9	0·10
,, ,, ,, ...	O, 21st transfer ...	1·95	0·07
Inverting sugar	I, 1st transfer	1·8	0·06
,, ,, ...	I, 23rd transfer ...	2·0	0·07
,, ,, ...	14, ...	3·7	0·13
,, ,, ...	23, ...	2·05	0·07
Acid sugar	30, 1st transfer ...	1·8	0·06
,, ,, ...	30, 21st transfer ...	1·75	0·06

It is evident from these results that the production of acid by the races is variable, and beyond the acidity being equal to between 0·06 and 0·13 % lactic acid, there is little to be inferred. Repeated cultivation of the races in the laboratory has no definite effect upon the acid-forming faculty, since in one case the culti vation has lessened the production, and in two others there is little difference. The amount of peptone in the nutrient fluid has a considerable influence upon the formation of acid, as was found in the experiments noted on page 596. With 0·01 % of peptone, the acidity on the fifth day was equal to 0·03 % lactic acid, while with 1 % of peptone the acidity was 0·18 %.

THE NATURE OF THE ACIDS.

In determining the nature of the acids a considerable number of cultures were made and tested. These were chiefly tentative or preliminary, and served to indicate the kinds of acids present and also the method which appeared to be best for their separation. Ultimately the following method was adopted :—

Two litres of the ordinary 10 % saccharose medium containing 100 grms. of chalk was infected with a mass culture (about 50 c.c.) of the organism and incubated at 37°. The culture was vigorously shaken every morning and the incubation was continued for a month, when it was noted that the evolution of gas bubbles had ceased. The culture was then treated in the manner described in the following table.

TREATMENT OF THE CHALK CULTURE.

<table>
<tr><td colspan="5">Two litres of culture incubated for 30 days with chalk; heated to 90°, filtered, washed.</td></tr>
<tr><td colspan="4">Filtrate evaporated to ⅓ volume, allowed to crystallise overnight, strained and pressed.</td><td>Residue, chiefly $CaCO_3$, decomposed with H_2SO_4; filtered, washed.</td></tr>
<tr><td colspan="2">Residue A.</td><td colspan="2">Liquor evaporated to ⅓ volume, allowed to crystallise overnight, strained and pressed.</td><td>Residue B</td><td>Filtrate C, added to G.</td></tr>
<tr><td colspan="2">Residues A, D decomposed with H_2SO_4; after standing overnight filtered and washed.</td><td>Residue D</td><td colspan="2">Liquor acidified with H_2SO_4; after some time filtered and washed:</td><td rowspan="2">Residues B, E, F were dried on porous tile, powdered and saturated with ether. After several days the ether was sucked off, evaporated, and water added to separate insoluble acid which proved to be Capric acid.</td></tr>
<tr><td>Residue F</td><td>Filtrate G had C added and the whole was extracted with ether from which the acids were separated.</td><td></td><td>Residue E</td><td>Filtrate distilled until ½ passed over.</td></tr>
<tr><td colspan="3"></td><td>Distillate contained Acetic and Formic acids.</td><td>Residual liquor extracted with ether chiefly lactic acid.</td><td></td></tr>
</table>

Before proceeding to describe the separation of the acids from the ethereal extracts a few notes may be made upon the organic acids mentioned in the table. The distillate from the mother liquor had a strong odour of acetic acid and was neutralised with soda. To a portion of the neutral solution silver nitrate was added, whereupon a pasty, white precipitate was thrown down. This was filtered, washed and dried on a porous tile, then *in vacuo* over sulphuric acid and finally in the oven at 80° until there was a loss of only 0·5 mgrm. in 30 minutes. A portion was dissolved in water and the silver estimated as chloride in the usual manner.

0·3645 grm. gave 0·3115 grm. AgCl = 64·33 % Ag.

Silver acetate contains 64·68 % Ag.

It is evident from the analysis of the silver salt that the acid is acetic.

The filtrate from the silver acetate rapidly darkened, which made it appear probable that formic acid was also present. Accordingly the neutralised distillate was tested and was found to reduce alkaline permanganate to the brown hydrated binoxide and also to produce a copious precipitate of calomel when treated with excess of mercuric chloride. The latter test is characteristic of formic acid.

From the calcium sulphate residues a few droplets of an insoluble fatty acid were obtained. The melting point was determined by a method which I devised some ten years ago. Two small particles of the dry solid acid are placed near one another on a coverglass, another coverglass is put on the top and very lightly pressed. The space between the particles after the second coverglass is in place should be from one to two millimetres. The preparation is then floated on mercury and covered with a small watch glass. The bulb of a delicate thermometer is inserted in the mercury and the temperature slowly raised. At the melting point the two particles of melting fat run together, the space between them disappears, and almost instantly the molten fat spreads out between the coverglasses. The melting point of

the separated fatty acid was 30·8° which is sufficiently near the melting point of capric acid (31·3°) to identify it with this acid.

The organic acids were extracted from the acid fluids by percolation with ether. The extraction was generally continued for 12 hours; sometimes with a large quantity of fluid the time occupied was 24 hours. After the extraction the ether was distilled off and the acids separated by a combination of the methods recommended by Schneider* and Harden.†

Analysis of the Ethereal Residue.

Ethereal residue diluted with water and distilled in a current of steam until the distillate had a constant acidity.

Residual liquor divided into portions.

Portion digested with ZnO, filtered while hot and the zinc lactate proved by its solubility and also microscopically.

Portion digested with $CaCO_3$ on water bath for several hours, filtered.

Residue dissolved in acetic acid and tested for oxalic acid: none found.

Filtrate evaporated to dryness, extracted with 90 % alcohol.

No residue of succinate

Extract contained lactate.

Portion of solution of calcium salt crystallised, dried and the calcium estimated.

Distillate evaporated with excess of $CaCO_3$, treated with absolute alcohol, filtered.

Residue extracted with water and solution tested for acetic and formic acids.
Acetic and formic acids found.

Filtrate.
Portion tested with $Zn(NO_3)_2$ for valeric acid; none found.
Remainder evaporated and residue tested for butyric acid.
Butyric acid found

Lactic acid was proved by the microscopical appearance of its barium, calcium and zinc salts, by its comparatively non-volatile

* Schneider, Journ. Chem. Soc. 1900, Abs. ii., 177.

† Harden, *ibid.* 1901, 614-5.

character (with steam), its action upon alkaline permanganate, its behaviour on dry distillation, Windisch's reaction and the analysis of the calcium salt. In the analysis the residue remaining after distilling off the volatile acids was heated on the water bath for several hours with excess of calcium carbonate, filtered, evaporated, and allowed to crystallise overnight. A solid mass of crystals was obtained which, after drying *in vacuo* over sulphuric acid, was dried in the water oven till nearly of constant weight. In one portion the calcium was estimated, and in another the water (by drying in water oven till of constant weight).

2·493 grms. contained 0·36 % of water and gave 1·1185 grms. $CaCO_3$ = 18·01 % Ca.

Calcium lactate contains 18·35 % Ca.

The butyric acid was separated as calcium butyrate by taking advantage of its comparative solubility in absolute alcohol. It was, however, never pure, being associated with formic and acetic acids. It was identified by the solubility of the calcium salt in absolute alcohol, by the pine-apple or rum odour of the ethyl ester, by the odour of the moist barium salt, and of the same salt when warmed with sulphuric acid. Finally the analysis of the silver compound prepared by the addition of silver nitrate to the mixture of substances obtained by dissolving the calcium salts in absolute alcohol while not absolutely agreeing with silver butyrate, is sufficiently distinctive (*i.e.*, low in silver) to show that butyric acid was contained therein. This silver compound, after precipitation and washing, was dried on a porous tile, and then at 80°, at which temperature there was no loss of water.

0·0548 grm. gave 0·0438 grm. Ag. Cl. = 60·16 % Ag.

Silver butyrate contains 55·38 % Ag.

Silver butyracetate contains 59·67 % Ag.

Silver acetate contains 64·68 % Ag.

With regard to the relative amounts of acids formed in saccharose solutions, a determination was made as follows. The acids separated from a fourteen days' chalk culture measured about 20 c.c. This was diluted with an equal volume of water, and distilled in a current of steam. When 141 c.c. had passed over the distillation was stopped, and a 10 c.c. portion was found to contain a quantiy of acid equivalent to 0·35 c.c. $^{N}/_{5}$ acid which for the whole 141 c.c. equals 4·9 c.c. The remaining 131 c.c. were evaporated down with chalk to dryness, and extracted with hot absolute alcohol. The calcium salt which dissolved weighed ·054 grm., and on the assumption that this consisted entirely of calcium butyrate, is equivalent to 0·048 grm. butyric acid in the whole distillate. The remaining acids (= 2·2 c.c. $^{N}/_{5}$), if taken as a mixture of equal parts of formic and acetic, would weigh 0·023 grm. This gives a total of 0·071 grm. of acids in the distillate. It must be borne in mind that lactic acid occurs in the distillate, and is calculated with the other acids, so that for this reason alone these figures must not be considered too narrowly. The main object in quoting them is to show how small is the quantity of the volatile acids when compared with the lactic acid. The residual fluid in the distilling flask was made up to 50 c.c., and 10 c.c. was boiled with zinc oxide and filtered. The filtrate, when evaporated and dried at 160°, yielded 1·076 grms. of a zinc salt. Another 10 c.c. was titrated with standard alkali by Kunz's method.* It contained—

Lactic acid	0·673 grm.
Dilactic acid as lactic acid ...	0·139 grm.
Total lactic acid	0·812 grm.

The zinc salts of these acids would have yielded 1·0745 grms., which agrees closely with 1·076 grms. found. In the total residual liquor there were, therefore, present 0·812 × 5 = 4·06 grms. lactic acid. Comparing this with the volatile acids, there

* Kunz, Journ. Chem. Soc., 1901, Abs. ii., 428.

is a ratio of 4·06 : 0·071 :: 57 : 1, and if some allowance is made for the lactic acid included in the volatile acids, it is evident that the bacillus forms about 60 times more lactic acid than volatile acids.

Mannite is not a Byproduct in the Gum Fermentation.

In the mucinous fermentation of beet juice by some bacteria, as, for example, *Leuconostoc mesenterioides*, the sugar is fermented partly to dextran, or fermentation-gum, of which there are two kinds, one a soluble form, the other an insoluble modification, and partly to mannite. In order to see if mannite was a by-product in the fermentation of sugar by this bacillus, several culture solutions were freed from gum, and after evaporation to a syrupy consistency, were extracted with strong hot alcohol. No mannite crystals could be obtained from any of the cultures. The syrupy residue of one, however, after standing for a week, contained a number of feathery tufts of needle-shaped crystals which might have been mannite. The whole syrup was dissolved in water and the sugars fermented out with a pure culture of *Saccharomyces cerevisiæ* I. The yeasts were then filtered off with the aid of aluminium hydrate. Mr. Steel kindly tested the rotation of the solution before and after the addition of borax. A very slight lævorotation was observed; the mannite, if present, was apparently in so small an amount that it could not be definitely determined. He subsequently tested a solution obtained from a culture containing 46 grms. of saccharose, and could find no evidence of mannite. It is, therefore, apparent that the bacillus does not form mannite from sugar. The crystals observed in the syrup were probably dextrose. Mannite was carefully tested for, because a gum-producing organism, *Bac. gummosus*, Happ, forms this hexatomic alcohol in sugar solutions.

Ordinary alcohol is likewise not a product of the fermentation. A litre culture was distilled with chalk, and in the first 50 c.c. of distillate, which had a sp.g. of 1·0008, no iodoform reaction could be obtained.

The Vitality of the Spores.

The spores of bacilli resist the action of moist heat at 100° C. for some time, and the gum bacillus is no exception to this rule. In testing how long they could withstand the action of boiling water, several tubes of saccharose media were infected with spores, and after attaching aerial condensers, the tubes were immersed in brine, which was kept slowly boiling. The media in the tubes boiled briskly. At intervals of $\frac{1}{2}$, $\frac{3}{4}$, 1, $1\frac{1}{2}$, $2\frac{1}{2}$ and 5 hours, tubes were taken out, cooled, and thereafter incubated at 37°. In all cases growth occurred and gum was formed. The spores can, therefore, withstand the action of boiling water for at least five hours.

According to Lafar, the potato bacillus can resist the influence of a current of steam for six hours, which marks it as being the most powerfully resistant of all organisms hitherto observed. The spores of the gum bacillus resist destruction as vigorously as the potato bacilli, and, as we shall see, this is not to be wondered at, for they have many points in common.

The Varieties of the Organism.

So far, with one exception, the action of a bacillus which was separated from roller cane juice has been considered. In the examination, however, of a number of raw and refined sugars, many races of the same organism were isolated. A few of these grew slowly, but otherwise they were identical with bacteria of quicker growth. From the more or less extended examination of some sixty bacteria, it became manifest that the races fell into one, two, or three groups, according to the value placed upon their growth characteristics. If they are divided into three groups, the third of these ($\beta\beta$ of table) must be considered as being derived from the second (β), since upon repeated cultivation the characters alter and become identical with those of β group. The β group differs from the α group chiefly in its method of growth in gelatine.

GENERAL CHARACTERS OF THE GROUPS

—	*a*	*β*	*ββ*
Agar stroke ...	Translucent white, amœboid layer, irregularly furrowed, dry or slightly moist, adhering firmly to the medium: often with glistening convex margin: the condensed water has a strong film.	White, amœboid, and sometimes terraced layer, dry and generally dull, adhering loosely to the medium, often with glistening, convex margin: condensed water with strong film.	Broad, glistening, yellow to buff, raised lobular growth: condensed water has no film.
Gelatine stab ...	Medium is liquefied very slowly as a small crateriform depression with white wrinkled film: stab at first filiform, then becomes arborescent (4-5 days).	Medium is liquefied quickly as a crateriform area. Then becoming saccate in 2-3 days, forms a loose surface film.	As *β*.
Potato	Reddish-white, dry, finely wrinkled growth on reddish ground: often with raised, apparently exuded, drops of watery fluid.	Pale yellow, deep yellow or yellow-brown raised undulating fatty growth, becoming dry and coarsely wrinkled.	As *β*.
Bouillon ...	Turbid, with strong wrinkled film.	As *a*	Turbid, but no film.
Milk	Curdled, then partly peptonised with faint acid reaction.	As *a*, but with alkaline reaction.	As *a*, with amphoteric or alkaline reaction.
Shape	Thin rods, 0·4-0·6 *μ* broad.	As *a*	Thick vacuolated rods over 1 *μ* broad.

The differences between *ββ* and *β* are very striking, and it would almost seem impossible that they could both be of the

same kind. Fortunately the alteration or reversion to the normal type occurred in the course of a few crops or transfers (erroneously called generations) with one of the races which was considered as being typical of group $\beta\beta$, and the identity of the groups was made evident. While with this race the change occurred by the 8th transfer, two other similar $\beta\beta$ races had not changed by the 40th transfer. From a glistening lobular yellow raised stroke, it altered to a dull white amœboid expansion spreading over the greater portion of the agar surface. Concomitant with this alteration, the granular or spongy structure of the bacterial protoplasm changed, and when stained the rods appeared narrow and homogeneous. The nature of the growth in bouillon, and on potato, also changed. In short, the race had become identical with those of group β. The reversion is shown in a somewhat diagrammatic form in the plate which accompanies this paper.

Why $\beta\beta$ should be so different from β can only be explained by assuming that it has been caused by the physical conditions that have obtained during the process of manufacture of the sugar. In the various samples of sugar the three groups of bacteria were generally found in varying proportions. This might point to the sugar having been infected at different periods of the manufacturing process, for if it were otherwise, only one group of bacteria would be found in each lot of sugar. As a rule, however, the prevailing bacteria were of the β kind.

The cultural variation of the race does not have much effect upon the production of gum. With one race of the $\beta\beta$ type, which maintained its character through 24 transfers, the amount of gum formed in 12 days at 37° was found to be 26·7 grms. for 100 grms. saccharose originally taken, the culture medium being identical with that already described.

Bacillus levaniformans, n.sp.

Shape, &c.—The films prepared from cultures on nutrient agar show the organism as a rod with rounded ends; the individuals occur singly and in chains. The dimensions of the rod vary con-

siderably, but, generally speaking, those races which produce white growths on nutrient agar (α and β) consist of cells measuring 0·4-0·6 : 2-3 μ. Occasionally a race of this normal type will have broader rods mixed with cells of that breadth. Those races which produce a buff colour on nutrient agar ($\beta\beta$) consist of a mixture of short, stout and of long rods varying from 1·3 : 2 to 1·0 : 6 μ. All cells stain readily, and are not decolorised by the Gram method. The broader cells appear to have a spongy structure, while the narrower cells stain uniformly. The rod forms a small oval and generally central endospore, readily with the white, but slowly with the buff races. Indeed, with the white races, the film on a 24 hours' bouillon culture at 37° consists chiefly of spores. Germination is lateral. The rods are motile, and in bouillon move about with a wriggling motion. In films of fluid saccharose media, the newly germinated rods have an active, darting motion. When films are prepared from a young agar culture, and stained by the night-blue mordant, as advised by Morton (Trans. Jenner Inst. ii.), the majority of the cells are seen to be capsulated, and to have many peritrichous flagella. Sketches of a few typical cells drawn with the camera lucida accompany this paper. The early cultures of the organism in fluid and sometimes in solid media, frequently show coccoid and streptococcoid swollen cells. These, however, have ceased to form in the second, third, or fourth transfer.

Relation to Oxygen.—There is practically no growth under anaërobic conditions. In Buchner's tubes, the stroke on agar is faint and amœboid, whilst bouillon becomes slightly turbid. In the fermentation tube, no growth occurs in the closed limb.

Agar plate.—Groups α and β: The colonies appear white, or grey-white, and are either flat and dry or raised and fatty. In some races the flat, dull colonies become amœboid and form a raised, moist, glistening margin, and the centre of the colony may become covered with watery globules. In other races the amœboid colony becomes wrinkled. Microscopically the deep colonies are ragged, woolly, or fibrous, the surface flat colonies are marbled or wrinkled, with a very irregular filamentous margin. The amœboid

processes are clouded and finely granular, with a clear central canal. Group $\beta\beta$: the colonies vary from dirty white to buff-white, and have a fatty or dull appearance. When magnified, the deep colonies are seen as irregular clumps; the surface colonies appear dark grey and marbled, with either a stippled or a filamentous margin, and a smooth or a rough edge.

Saccharose(10 %)-*peptone*($\frac{1}{10}$ %)-*agar plate.* — The colonies of the α and β types appear like raised drops of whey upon an amœboid base. When magnified, the deep colonies are lenticular and clouded; the surface colonies being clouded and hatched. The amœboid processes of the surface colonies have a hatched centre, a finely granular intermediate portion, and a clear margin. The colonies of the $\beta\beta$ type are circular and yellowish, or dirty white. The agar below the surface may swell into a dome-shaped prominence bearing the colony. Subsurface colonies cause the agar to swell locally and split.

Agar stroke.—Group α: The races form a translucent white amœboid growth spreading over the greater portion of the agar surface. The growth becomes irregularly furrowed or indented, and appears either dry or slightly moist. Sometimes the surface is covered with minute drops of fluid, which gives the culture a shagreen appearance. The upper margin of the stroke is frequently raised and glistening. The central portions adhere so firmly to the agar that it is impossible to remove portions with the ordinary thin platinum needle. The condensed water is covered with a strong, white, wrinkled film. Group β: The cultures are whiter than the α races, and show a greater diversity of appearance. The growths are amœboid, and either flat or terraced. They may be dry or glistening. A white central portion may have a narrow or broad raised translucent margin. Some races differ from those of the α group only in having a fatty consistence, so that portions of the culture can be easily removed with the needle. Like the α group, the cultures form a strong, white, wrinkled film on the condensed water. Group $\beta\beta$: The growth is broad, raised, luxuriant, and varies in colour from primrose through stone to buff. It is lobular and spreading, but

never amœboid. It appears either moist, glistening, or fatty, and the consistency is soft like that of butter. The condensed water generally has a flocculent yellowish sediment, but never has a film.

Saccharose-peptone-agar stroke.—With all groups, there is formed a broad convex ridge, undulating as if containing gelatinous lumps. The growth is translucent and indistinguishable from the medium. The consistency is gelatinous.

Gelatine plate.—Group *a*: The colonies grow slowly; on the third day they are white and punctiform. When magnified, the deep colonies are seen to be round, moruloid or like woolly tufts, with outstretching fibres interspersed with cellular clavate processes. The surface colonies are rounded erose, with a granular centre and clouded margin. On the fourth day the surface colonies appear depressed and are seen, microscopically, to be deeply pitted and wrinkled, and to have a rough margin. On the sixth day the surface colonies consist of a dry, wrinkled film, with a central white point. There is a slight softening of the gelatine, and in crowded plates the medium becomes slimy. Groups β and $\beta\beta$: White circular crateriform liquefied areas containing a white sediment are formed in two days. Microscopically the sediment consists of coarse granules, and the margin of the colony (liquefied area) is ciliate. The deep colonies appear circular and either opaque or coarsely granular, with a ciliate edge.

Gelatine stab.—The stab is primarily filiform, with a slight depressed surface growth. In from four to six days, the needle track is beset with coarse white arborescent outgrowths, and the gelatine at the surface is slightly liquefied and consumed. The liquefied medium is covered with a deeply wrinkled film. Groups β and $\beta\beta$: The growth is at first white and filiform, but the medium is sooner or later (1-2 days) liquefied, the top of the stab becoming funicular; finally the entire stab becomes saccate. There is formed a thin surface-film.

Bouillon.—Groups *a* and β: The medium becomes faintly turbid and covered with a strong but easily detachable wrinkled white

film. Group $\beta\beta$: The bouillon becomes turbid and forms a white sediment and a very slight surface-ring. Indol is formed by all the groups, and nitrate may be slightly reduced to nitrite.

Milk.—All groups coagulate and then slowly peptonise the casein, but group α is much slower in its action. The reaction of the supernatant liquid may be faintly acid (α), amphoteric ($\beta\beta$), or alkaline (β).

Peptone-saccharose fluid.—The white, milk-like appearance is very characteristic.

Potato.—Group α: There is formed a reddish-white thin layer upon a reddish ground. The thin layer becomes folded into small delicate wrinkles. Sometimes in place of wrinkles drops of a white watery fluid exude from spots scattered over the apparently red surface of the potato. Group β: The cultures are of various shades of stone, yellow or yellowish-brown. They are raised and undulating and soon (3-4 days) become coarsely wrinkled. Group $\beta\beta$: The growths are similar to β, but in addition the medium is frequently darkened in the vicinity of the culture.

Temperature.—The organism grows at 15°, 22°, 30°, and most quickly at 37°; above 37° the growth rapidly diminishes; with the exception of the cultures in gelatine, which were made at 22°, the descriptions of the growth characters apply to cultures at 37°.

The Gum-forming Bacilli previously Described.

From the literature at my disposal upon this subject I have found notes upon a number of sporeless bacteria capable of converting saccharose into gummy substances when natural media, such as slices of beet, were infected. As, however, the organism separated by me is a spore-forming bacillus, mention of other spore-bearing organisms which are known to form gum from saccharose need only be made.

Fritz Glaser* separated an actively motile short rod-shaped bacterium, *Bact. gelatinosum betæ*, from beet juice that had undergone a mucinous fermentation. It rapidly liquefies gelatine, produces gas when grown in beet juice at 40-45° C., and is not

* Fritz Glaser, Cent. f. Bakt., 2te Abt., i. 879.

killed when heated to 100° C. This fact would indicate the presence of spores, but the author apparently never observed spore formation. The organism also inverts saccharose, forming a quantity of alcohol but no lactic acid. According to Lafar* the alcohol is amyl alcohol. From but a few tests, Glaser considered the gum to be probably identical with Scheibler's dextran or beet gum.

Ritsert† separated from a slimy digitalis infusion an organism, *Bact. gummosum*, which formed gum in saccharose solutions containing nutrient salts such as potassium acetate and ammonium phosphate. Similar solutions of grape sugar or of milk sugar did not produce gum after infection. In the saccharose cultures nearly half of the sugar was converted into gum, which was estimated by precipitation with alcohol and weighing. Besides the gum, which he named gummose, an acid and a dextro-rotatory body capable of reducing Fehling's solution were formed. The organism was an anthrax-like rod which formed threads, chains of rods, streptococcus, diplococcus or coccoid forms according to the medium in which it was growing. It formed endogenous oval spores and the rods were not stained when treated by the Gram method. The growth on agar was lobular and glistening white; after a few days it showed two zones—the inner wrinkled, raised, dry and white; the outer glistening and bluish-white. In bouillon the rod was feebly motile. Alkaline gelatine was liquefied.

Happ‡ also separated from digitalis infusion a bacillus, *Bac. gummosus*, capable of producing a gummy fermentation in vegetable infusions. It appeared as a large thick rod with rounded corners, measuring 0·6-2 : 5-7·5 μ. It was weakly motile; flagella and spores were observed. The colonies on gelatine were at first circular, then processes were sent out into the medium which quickly liquefied. In stab culture the liquefaction of the gelatine was funicular. On agar, the stroke became a moist glistening and characteristically lobular (? amœboid) growth. On

* Lafar, Technical Mycology.

† Ritsert, Cent. f. Bakt. 1te Abt., xi. 730, Ref.

‡ Happ, Cent. f. Bakt., 1te Abt., xiv., 175, Ref.

potato, coccoid involution forms were noted on the second day. On this medium the growth is at first moist glistening, but after some weeks it became a whitish folded skin adhering firmly to the medium. The optimum temperature lay between 25° and 30°. The presence of saccharose is absolutely necessary for the formation of gum; no other sugars induce the mucoid fermentation. Saline substances are favourable to the fermentation, but these are not absolutely essential, since in their absence sugar solutions can form gum. Albuminoids are likewise not absolutely necessary in the fermentation. The by-products are mannite, lactic acid, butyric acid and carbonic acid. Only a small part of the saccharose is converted to dextrose.

The Affinities of the Bacillus.

Fritz Glaser has not described his bacterium at all fully, and he did not investigate his gum with any detail, but from the fact that it forms (amyl?) alcohol and no lactic acid it is evidently different from that which I have investigated.

Both Ritsert's and Happ's organisms are like one another. They were separated from the same kind of infusion, they show a similar variability of form according to the medium; they produce acids and they invert saccharose. With regard to the inversion, Ritsert has not described the product of inversion otherwise than as a dextro-rotatory body capable of reducing Fehling's solution, while Happ said that only a small part of the saccharose was inverted. The byproducts in the fermentation of Happ's bacillus are mannite, lactic acid, butyric acid and carbonic acid. Ritsert did not identify his acid, and does not mention mannite. I can find no mention of the nature of Happ's gum beyond its being soluble in water, insoluble in alcohol and ether, and having the composition $(C_6H_{10}O_5)_n$. Ritsert's was optically inactive.

Although one cannot identify these two organisms by their enzymic activities, it is possible from their cultural characters that they are varieties of one common type, and that is possibly *Bac. vulgatus*, to which my organism is probably also related.

There are, however, many points of difference between the chemical products of my bacillus and those of Ritsert and Happ. Since it is the products which mark the importance of the organism, and since these are different to the products of bacteria already described, I have thought it advisable to give the organism the distinctive name of *Bacillus levaniformans*. At the same time it must be borne in mind that it is probably a variety of *Bac. vulgatus* which, together with several other allies, constitute the potato group of bacteria. The members of this group of bacteria have many points in common; in fact there are so many common characters that what are at present called species are undoubtedly only races. To show this I shall enumerate shortly the characters of these species as described by Lehmann and Neumann, and it will be seen that *Bac. levaniformans* has affinities with all the members of the group.

The Mesentericus Group of Bacilli.

Bacillus (mesentericus) vulgatus occurs as a thin rod, with slightly rounded ends, measuring $0{\cdot}8 : 1{\cdot}6\text{-}5\,\mu$. It frequently forms threads, and easily produces plump oval spores. The rods are motile and are studded with flagella. The bacilli are stained by Gram's method. On gelatine plates there are formed crateriform liquefied areas covered with a delicate whitish folded film. In gelatine stab culture there is a crateriform liquefaction over a filiform growth. On agar plate, there appear whitish, moist glistening, raised, smooth or rough-edged colonies. When magnified the centre of the colony appears homogeneous without markings, and the margin is often filamentous; the deep colonies are homogeneous and frequently ciliate. The agar stroke is raised, lobular, fatty and grey-white; after some time it becomes folded and the condensed water is covered with a strong film. Bouillon is made turbid and forms a strong grey-white film; no indol is formed. Milk is curdled and has a slimy consistency; the reaction is strongly alkaline. The potato cultures are most variable. The typical growth is raised and irregularly swollen like the mesentery, whence its general name, *Bac. mesentericus*

vulgatus. The colour is partly whitish-grey, partly yellowish or yellow or even reddish-brown. The culture finally covers the potato as a slimy mass.

Bacillus mesentericus (fuscus) differs from *vulgatus* chiefly in colour and in not liquefying gelatine so quickly. The liquefied gelatine in stab culture has a surface film. The stroke on agar is yellow-brown. On potato the growth is raised, moist glistening, greyish-yellow and slimy, afterwards becoming a dull raised network. Traces of indol are formed in bouillon.

Bacillus liodermos differs from *vulgatus* in forming on potato a smooth glistening yellowish-white syrupy growth which is soluble in water and precipitated from solution by alcohol.

Bacillus mesentericus ruber is very similar to *vulgatus.* It forms colonies of various shape on gelatine which is slowly liquefied. The agar-stroke is translucent, grey-white, moist glistening, and eventually becomes like a network. The growth on potato is mesentery-like and of a rose-red colour which ultimately becomes reddish-brown.

It is quite probable that the four members of the potato group are all varieties of one typical bacterium, which we may assume to be that most commonly occurring, viz., *vulgatus.* The others differ but slightly from it. Examples of a similar variation are not wanting among the bacteria. Among the yeasts the varieties of *Sacch. cerevisiæ* appear to be limited only by the number of breweries. Conn noted the occurrence in nature of a white coccus, varieties of which did or did not liquefy gelatine. Schierbeck* separated from one sample of milk races of lactic bacteria which differed in their fermentative power, and moreover the difference was apparently fixed so far as the race was concerned.

But coming to the *vulgatus* group itself, there are instances where one or other of the members have developed the power of causing bread to become slimy. Uffelmann† ascribed the

---

* Schierbeck, Cent. f. Bakt. 1te Abt., vii. 239, Ref.

† Uffelmann, Cent. f. Bakt. 1te Abt., viii.. 481.

exciting cause of slimy rye-bread to *Bac. vulgatus*, after separating from the bread that organism and *Bac. liodermos*. Juckenack* separated *Bac. mesentericus* in pure culture from a batch of ropy rye-bread. Eccles† found the slimy fermentation quite commonly in the bread of certain localities when it had been kept warm for some time after baking. He traced the fermentation to *Bac. vulgatus* and *Bac. liodermos*, the latter producing a greater degree of sliminess than the former. We thus have cases of a particular kind of fermentation—the alteration of the crumb of bread into a gummy or ropy mass—induced by *vulgatus*, *mesentericus* and *liodermos*. This assumption of a common function by these so-called species when considered with the fact that their cultural characters are not very distinctive is enough to indicate the probability of the species being really races of one bacillus.

Ritsert's *Bac. gummosus* and Happ's *Bact. gummosum* do not apparently differ greatly from *Bac. vulgatus*, and may well be races. Lehmann and Neumann consider that they are allied to that bacillus.

With regard to the races which were separated in this research, they appear to be allied not to any particular member but to the group as a whole. The race which was separated from cane juice and which I have called the *a* type, appears, if the growth on potato is an index, to be related to *Bac. mesentericus ruber* as well as to *Bac. vulgatus*. The arborescent growth in gelatine stab culture is peculiar to that race, and does not occur among races which are identical in every other respect. The races of the derived type are closely related to *Bac. mesentericus* so long as the cell protoplasm remains granular or spongy. Once the protoplasm condenses, as it may do by repeated cultivation in artificial media, the colour, the appearance and the consistence of the growth upon agar also change, and the race becomes related to *Bac. vulgatus*, but still differs from it by reason of the spreading, amœboid nature of the agar culture. The races when grown

* Juckenack, *ibid.* 2te Abt., vii., 109, Ref.

† Eccles, Jour. App. Microscopy, iv. (3), 1222, Abs,

on agar show practically every variation from a slight, almost transparent, watery smear to the luxuriant, corrugated, amœboid, tough layer over most of the agar surface.

The Source of the Bacillus.

It must not be presumed that the organism is derived from the gum of "gummy cane," *i.e.*, plants affected with gummosis. It can be affirmed without a shadow of doubt that it does not, for the reason that the gum which is found in gummed canes is absolutely different from the gum formed by the bacillus. At one time I thought it just possible that a different medium might produce a different gum, and grew the bacillus in plant infusions (*e.g.*, hay, turnip) with sugar, but the gum produced in such infusions was found to be identical with that formed in the peptone solution. We can, therefore, say that the bacillus produces only one kind of gum, viz., levan, which is absolutely different from cane gum.

The microbe has been separated from cane juice and from refined sugars, and since it occurs in these two places—the beginning and the end of the manufacturing process—it will certainly be found in all positions in the factory and the refinery. It undoubtedly obtains access to the factory with the cane,* most probably on the outer surface of the plant. And having once got into the manufactory, and being in congenial surroundings, it will thrive luxuriantly unless means are adopted to check its growth. Experience has taught the manufacturer that the best preventative for gumming is to push on the crystallisation of the sugar as quickly and at as high a temperature as possible.

---

* The bacillus was obtained from the interior of a portion of gummy cane from Fiji, but as the portion had been lying about the benches in the laboratory of the Colonial Sugar Refining Co. before it came into my possession, there is the possibility that the organism obtained access to the centre of the cane during that time. I sought for the bacillus in five samples of gummy cane from New South Wales, and found it in the tissues of one of the samples.

To this may be added the hackneyed suggestion of disinfecting the premises and plant whenever possible.

If the manufacturer desired to produce gum, he would use such a saccharine fluid as cane juice, and after clarification infect it with the bacillus. Then he would keep the juice as near 37° C. as possible, but not much over that temperature, and certainly not over 44° C. The juice would also be well aerated by tossing or spraying, and finally the fermentation would be allowed to go on until the maximum amount of gum had been formed. Repeated infection during the process, as by running the juice into dirty vessels, &c., would greatly assist the production. With this knowledge of how to best produce gum, it is easy to see how the formation can best be prevented.

To the intelligent manufacturer the knowledge of the cause is enough to give the cue for its attempted elimination. Whether this is possible, experience alone can show. Were it not for the constant infection from without by the introduction of infected material, the trouble might be overcome. Probably all the manufacturer can hope for is a diminution of the gumming. The microbe is exceedingly hard to kill. The vegetating forms must be, to a certain extent, protected, during a part of their life at least, by the gum capsules, and the spores can resist the action of boiling water for at least five hours. But difficult as the task is, it must be attempted so that the manifold changes which the microbe induces may be minimised or prevented.

EXPLANATION OF PLATE.

The figures illustrate diagrammatically stages in the conversion of the derived type of *Bacillus leraniformans* (right figures) to the normal type (left figures). The medium, the number of the transfer or crop, and all colours except white are noted. Shading indicates a dull appearance, absence of shading shows that the culture had a glistening appearance when examined. The two types were inoculated, incubated, and observed side by side. One-half natural size.